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IN SITU HYBRIDIZATION TO DETECT PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

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Abstract:

Porcine reproductive and respiratory syndrome (PRRS) has for nearly 3 decades been economically one of the most important swine diseases. Despite intensive research focus, many unanswered questions remain

regarding the pathogenesis of PRRSV. *In situ* hybridization (ISH) is generally considered a more useful diagnostic tool than immunohystochemistry (IHC) and may be helpful in further research ofpathogenesis. ISH is able to detect virus in non-progressive stages therefore the length of successful detection after infection is expected. It is not widely used, however, because of problems with specificity of the oligonucleotide probe due to the pronounced diversity of the PRRSV genome. The aim of the present study was to evaluate a PRRSV specific ISH protocol, Three, non-overlapping PRRSV specific 20 nucleotides , DIG labeled oligonucleotide probes were designed targeting the ORF7 region. The probes were specific designed to recognize PRRSV Type I isolates only. A total of 19 positive PRRSV paraffin blocks from different organs and infected with different strains were tested as well as a negative control. All samples were simultaneously tested by IHC using different anti-PRRSV monoclonal antibodies. Five experiments of ISH were performed, using a pool of 1 nmol of each of the three oligonucleotide probes with two

different prehybridization temperature (105°C and 80°C) and time (5 and 10 min), using 0.5 nmol of each of the probes separately with prehybridization on 105°C during 5 min. Positive signals were detected in alveolar macrophages in lungs, in hystiocytes in lymph nodes, Payer patches and tonsils, in macrophages, on inflamed area in ileum and in glomerular cells. 58 EuroPRRS2012 Budapest, Hungary ISH showed better sensitivity than IHC while there was an obvious discrepancy between sensitivity among the probes.

Key words: Porcine reproductive and respiratory syndrome, *in situ* hybridization

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